## Interim Guidance for Brucellosis testing within the three Greater Yellowstone Area States FPA > BAPA Interpretation and Herd Disposition/Follow-up

<u>Subject</u>: Re-ordering of brucellosis test application in series for screening purposes

## Discussion

Due to the unavailability of RAP antigen, standard high-volume testing protocols in the three Greater Yellowstone Area (GYA) states was disrupted in the fall of 2019. Conducting the BAPA as the primary screening method in GYA laboratories was not logistically feasible due to speed of commerce and the need for rapid test results. Therefore, the GYA States in partnership with Veterinary Services adjusted the protocol to apply the FPA as the initial screening test to better meet testing volume needs. Previously under the National Standardized Brucellosis testing protocol only animals with non-negative RAP or non-negative BAPA results were tested with FPA.

The FPA plate test is a much more sensitive test than the BAPA or RAP and has the potential for increased false positives at the initial screening phase. This has been observed in the GYA states this fall where false positive rates are increased compared to previous years. Without a viable second confirmatory test in series to balance the specificity, the program is left with a much less specific protocol that affects producers more often and disrupts the speed of commerce.

On November 22<sup>nd</sup>, VS shared with the National Assembly of State Animal Health Officials a draft protocol to address increased false positives that did not include a second confirmatory test in series to balance specificity. After further discussions with the National Veterinary Services Laboratory and the three GYA States, VS determined that BAPA should be applied as the second test in series and adjusted the protocol further, presented here.

This GYA protocol would apply the FPA plate as the initial screening test with the BAPA test in series as a confirmatory test in order to maintain acceptable specificity. This new protocol would have the exact same calculated specificity (1 - rate of false positives) as the previous BAPA >> FPA >> CF protocol. Complement fixation would be applied as before as a supplemental test on all confirmatory test positives. The main difference in this protocol is that the most sensitive test (FPA) is applied first.

It is important to note that if the FPA and BAPA were just simply reversed in testing order but still interpreted in series that the combined specificity is exactly the same. It doesn't matter if you multiply 5X2 or 2X5, the product is still the same... so the calculated combined sensitivity 93% and specificity 99.97% of this new protocol is exactly the same as it was before. We are simply using the more sensitive test first. Additionally, since implementation of the previous longstanding National Standardized Brucellosis testing protocol in 2013, the GYA has not allowed a positive animal to escape the GYA while maintaining tolerable specificity to support the speed of commerce.

FPA Plate Screening	Classification (with BAPA & CF)	Herd Disposition
Delta mP value (delta mP 1-11)	Negative No BAPA needed	No Action Required
Delta mP value  If (11< delta mP ≤40), run the BAPA as confirmatory test.	Negative  If BAPA (-), then classify case as negative. No CF needed.	No Action Required
Delta mP value  If (11< delta mP ≤21), run the BAPA as confirmatory test.	Suspect  If BAPA (+), then run FPA Tube and CF for additional epidemiologic info	Local NAHLN lab BAPA result acceptable Suspect is investigated Epidemiologist can classify as negative based on investigation Herd is not quarantined. DSA rules still apply*
Delta mP value  If (21< delta mP ≤40), run the BAPA as confirmatory test.	Suspect  If BAPA (+), AND CF test results are negative  Run FPA tube and CF on BAPA (+)s @ NVSL	Local NAHLN lab BAPA result acceptable Suspect is investigated and retested Herd is not quarantined. DSA rules still apply*
Delta mP value  If (21< delta mP ≤40), run the BAPA as confirmatory test.	Positive  If BAPA (+), AND CF test results are positive, anticomplementary or unavailable.  Run FPA tube and CF on BAPA (+)s @ NVSL	Local NAHLN lab BAPA result acceptable Normal epidemiological investigation proceeds and Positive is retested Herd is quarantined**
Delta mP value  If (delta mP >40), run the BAPA as confirmatory test.	Positive  If BAPA (+), regardless of CF test results  Run FPA tube and CF on BAPA (+)s @ NVSL	Local NAHLN lab BAPA result acceptable Normal epidemiological investigation proceeds Herd is quarantined**
If (delta mP >40), run the BAPA as confirmatory test.	Consult RHC and SAHO  If BAPA (-), then consider epidemiology and consult RHC and SAHO	This will require normal epidemiological investigation. Movement restrictions, quarantines and follow-up testing only on case-by-case basis.

Classification Table for FPA screening followed by FPA and CF for us in GYA testing

## **Final Notes:**

Send protocol positive cases to NVSL for confirmation:

- Positive BAPA + non-negative FPA = send to NVSL for confirmation
  - o NVSL will run FPA and CF if sample is a GYA submission.
- FPA plate non-negative + positive BAPA or unavailable = send to NVSL for confirmation
  - Local labs can run CF but still send sample to NVSL for confirmation regardless of local CF results
  - If BAPA wasn't run locally, NVSL will screen with BAPA and if negative would stop there. If BAPA positive, then NVSL will run FPA and CF.